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EXPERIMENT K-6-20

THE EFFECT OF SPACEFLIGHT ON PITUITARY OXYTOCIN AND VASOPRESSIN CONTENT OF RATS

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SUMMARY

Pituitary levels of oxytocin (OT) and vasopressin (AVP) were measured in rats exposed to 12.5 days of spaceflight (FLT) as well as ground-based controls, one group synchronously maintained in flight-type cages with similar feeding schedules (SYN), and one group in vivarium cages (VIV). Flight rats had significantly less ($p< 0.05$) pituitary OT and AVP (1.10 ± 0.04 and 1.69 ± 0.07 µg, n=5) than either the SYN (1.50 ± 0.08 and 2.11 ± 0.04 µg, n=5) or VIV (1.54 ± 0.03 and 2.10 ± 0.09 µg, n=5) control groups, respectively. Because the FLT group mean body weight was significantly less ($p< 0.05$) than either control group, the pituitary hormone content was also calculated on the basis of posterior pituitary protein content (µg hormone/mg protein). When calculated in this manner pituitary OT in the FLT rats (5.09 ± 0.15 µg/mg protein) was significantly less ($p <0.05$) than SYN (7.66 ± 0.39 µg/mg protein) or VIV controls (8.11 ± 0.64 µg/mg protein). Pituitary AVP was also less in the FLT animals (7.80 ± 0.13 µg/mg protein) compared to either SYN (9.84 ± 0.51, $p< 0.05$) or VIV controls (11.01 ± 0.76, $p< 0.05$). The reduced levels of pituitary OT and AVP may have resulted from increased hormone secretion resulting from the combined effects of water deprivation and the stress of the novel microgravity environment.

INTRODUCTION

Disturbances in fluid and electrolyte balance have been noted in humans exposed to spaceflight (Leach and Rambaut, 1977). This was shown by a loss of plasma volume and increased excretion of sodium and potassium during flight (Leach and Rambaut, 1977). Upon return to earth these imbalances are quickly corrected with rehydration and increased renin-angiotensin-aldosterone activity (Leach and Rambaut, 1975). Similar postflight fluid-electrolyte and hormone responses have been observed in rats that were exposed to microgravity (Gazenko, et al., 1984). Microscopic examination of the hypothalamus and posterior pituitary gland of flight rats exposed to spaceflight shows changes indicative of increased activity e.g. increased hormone synthesis and secretion (Savina, et al., 1976). The purpose of this investigation was to measure levels of pituitary oxytocin (OT) and vasopressin (AVP) as possible indicators of changes in fluid-electrolyte balance during spaceflight.

METHODS

Animals

Male, specific pathogen free Czechoslovakian-Wister rats were divided into three groups of 10 animals each. Each group was housed in a single cage equipped with 10 paste food dispensers and 10 water lixits. All animals were fed a special paste diet before flight (Grindeland, et al). The flight group (FLT) was selected and transported, with a second group that was designated the synchronous control group (SYN), to the launch site. The third group remained in Moscow and served as the vivarium controls (VIV). The SYN animals were exposed to centrifugation and vibration to simulate the increased gravity associated with launch. The SYN animals were fed on the same schedule as the FLT rats. The VIV rats received an equalivant amount of paste diet each day, but it was dispensed in a single bolus at a fixed time each day. Water was available to all animals ad lib except during reentry and recovery. Five rats from each group were available for posterior pituitary measurements of OT and AVP. Further details concerning the care and treatment of the flight and control animals during flight and recovery are presented elsewhere (in this issue (Grindeland, et al).

Collection of Pituitaries

The animals were sacrificed at various times after recovery (Grindeland, et al.). Immediately after decapitation, the skull was opened and the brain was carefully removed to expose the anterior and posterior pituitary. The posterior pituitary along with the intermediate lobe was teased free from the anterior lobe, placed on a small square of aluminum foil, and frozen by immersion in liquid nitrogen. The foil containing the pituitary was placed in a small cyrovial and reimmersed in liquid nitrogen. The vials were transported and maintained on dry ice until they were thawed immediately prior to homogenization. The pituitaries were thawed individually and homogenized in 1 ml of 0.1 N HCl. An aliquot of the homogenate was diluted 1:200,000 in 0.05M phosphate assay buffer for radioimmunoassay of OT and AVP (Keil, et al., 1984; Keil and Severs, 1977). To eliminate interassay variability aliquots from all three groups (FLT, SYN, and VIV) were measured within the same OT or AVP radioimmunoassay. After the hormone levels were determined, protein concentrations were measured in aliquots from each homogenate (Pierce BCA. protein assay , Pierce Chemical Co.). Hormone concentrations were then calculated as a function of total protein for each posterior pituitary homogenate. Statistical comparisons were made among the various groups with a one-way ANOVA and the Newman-Keul range statistic as well as the nonparametric Mann-Whitney U test.

RESULTS

The assay results are expressed as micrograms of hormone per pituitary (Fig. 1) and as micrograms of hormone per mg protein. (Fig. 2). The latter calculation was made to provide a more valid comparison of FLT data with controls because the average weight of the FLT animals was significantly less than either control group. The body weights (g ± SE) of the rats at the time of sacrifice were: FLT. 303 ± 2; SYN. 349 ± 6; and VIV. 342 ± 8.

Both neural lobe hormones levels were significantly reduced in the FLT animals when compared to either set of controls by either parametric or nonparametric tests. Oxytocin was 31% lower, 1.10 ± 0.04 µg/pituitary ($p<0.05$) in the FLT group compared to 1.60 ± 0.08 the SYN rats, and 29% lower, 1.54 ± 0.03 µg/pituitary ($p<0.05$) in the VIV controls (Fig. 1). Pituitary AVP in the FLT animals (1.69 ± 0.07 µg/pituitary) was 20% lower ($p<0.05$) than either of the control groups, 2.11 ± 0.04 SYN and 2.10 ± 0.09 VIV (Fig 1.). Because the FLT animals weighed less than either set of controls, the pituitary hormone content was calculated on the basis of protein content (µg hormone/mg protein) in posterior pituitary homogenate. The results are shown in Figure 2.

When expressed in terms of pituitary protein content, the results still indicate a significant reduction in pituitary OT and AVP compared to either control group by both parametric and nonparametric tests (Fig. 2). Pituitary OT in the FLT group was 33.6% lower (5.09 ± 0.15 µg/mg protein) compared to 7.66 ± 0.39 for the SYN controls ($p<0.05$) and 37.3 % lower (8.11 ± 0.64) than in VIV controls ($p< 0.05$). Pituitary AVP was 20.7% lower (7.80 ± 0.13 µg/mg protein) in the FLT group compared to that in SYN controls (9.83 ± 0.51, $p<0.05$) controls and 29.2% lower (11.01 ± 0.76) than that in the VIV controls ($p<0.05$).

DISCUSSION

Postflight investigations of both humans and animals have indicated that fluid-electrolyte balance is altered by exposure to microgravity (Leach and Rambaut, 1977; Gazenko, et al., 1984). Post-flight reductions in sodium excretion and urine volume, and increases in plasma renin activity are indications that changes in fluid-electrolyte homeostasis occurred during flight (Leach and Rambaut, 1975). In humans, the headward fluid shift observed during flight is thought to be the stimulus for the plasma volume reduction and other adjustments in fluid-electrolyte metabolism. Rats and other horizontal animals may not experience this headward fluid shift, and if such a shift did take place, it may be proportionally less than that of humans because of the difference in

hydrostatic columns. However, results from rat postflight experiments are similar to those of humans, e.g. sodium and water excretion reduced and water consumption increased (Gazenko, et al., 1984). These postflight results demonstrate that changes in fluid-electrolyte balance have taken place during flight. Whether the same physiological mechanisms are responsible for these changes in rodents remain to be determined.

In the present study, FLT rats had significantly less pituitary OT and AVP than either group of ground-based controls. Decreased content may be attributed, in part, to the difference in size between the flight and control groups. The mean body weight of the FLT rats was 40 to 50 g less than that of either control group. A reduction in body size indicates that the FLT animals did not maintain the same growth rate as that of the controls, and this would result in decreased pituitary size and hormone content. Since the SYN controls consumed the same amount of food as the FLT rats, the decrease in growth of this group cannot be attributed to reduced food intake.

In an effort to compensate for the difference in body weight between the groups, hormone content was calculated on the basis of pituitary protein content (Fig. 2). In this case, AVP content of the FLT rats was 20.7 and 29.2% lower than either SYN or VIV control groups, respectively. This indicates that the FLT animals may have been dehydrated. Synchronous controls had access to water on the same schedule as the FLT animals; however, the pituitary OT and AVP content of the SYN rats was sigificantly greater than that of the FLT group. This suggests that the FLT rats had water available, but for some reason did not drink or, alternatively, the SYN group had water available for a longer period of time or drank more than the FLT animals.

Pituitary OT was also significantly lower in FLT rats (Figs. 1 & 2). Dehydration reduces pituitary OT as well as AVP (Summy-Long, et al., 1984). In addition, data from recent papers indicate that OT secretion, at least in rats, is increased by stress (Gibbs, 1986a). Oxytocin facilitates the action of Corticotrophin Releasing Factor (CRF) on the anterior pituitary to stimulate the release of Adrenocorticotrophic hormone (ACTH) (Gibbs, 1986b). Gibbs (1986a) also points out that OT may be released in response to "neurogenic" stress, whereas AVP, which also has been shown to increase CRF activity, is released in response to "physical" stress. Perhaps some of the decrease in pituitary OT may be attributed to the chronic neurogenic stress the animals experienced during flight in their effort to adapt to microgravity. This OT response to stress may be unique to rats because a similar response has not been observed in humans (Legros, et al., 1987).

In rats, administration of nausea-producing agents such as lithium chloride and apomorphine results in learned taste aversion as well as a relatively specific release of OT rather than AVP (Verbalis, et al., 1986). Increased plasma levels of the gut hormone, cholecystokinin (CCK), are also associated with taste aversion and nausea (Verbalis, et al., 1986). This hormone appears to be a specific stimulus for the secretion of OT, in rats (Carter and Lightman, 1987). Interestingly, lesions of the area postrema, a circumventricular organ associated with nausea and the emetic response, signifcantly reduced OT secretion in response to CCK injection (Carter and Lightman, 1987). If the rats experienced motion sickness during flight, it is possible that this condition, along with stress and dehydration, would contribute to increased OT secretion and depletion of pituitary OT stores.

In summary, the results indicate that the FLT animals were dehydrated and this led to a significant reduction in both pituitary OT and AVP. Results also show that pituitary OT was reduced to a greater extent than AVP, and perhaps this decrease was in response to increased stress or motion sickness encountered during flight and/or recovery.

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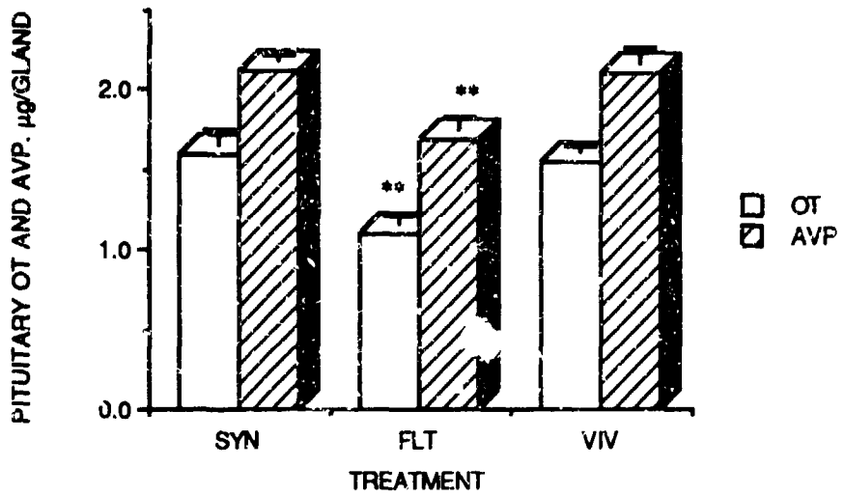

Figure 1. Pituitary oxytocin (OT) and vasopressin (AVP) content expressed as µg per posterior lobe. **p<0.05 for comparison of Flight (FLT) to either Synchronous (SYN) or Vivarium (VIV) control by both the ANOVA or Mann-Whitney U tests. Values are means ± SE.

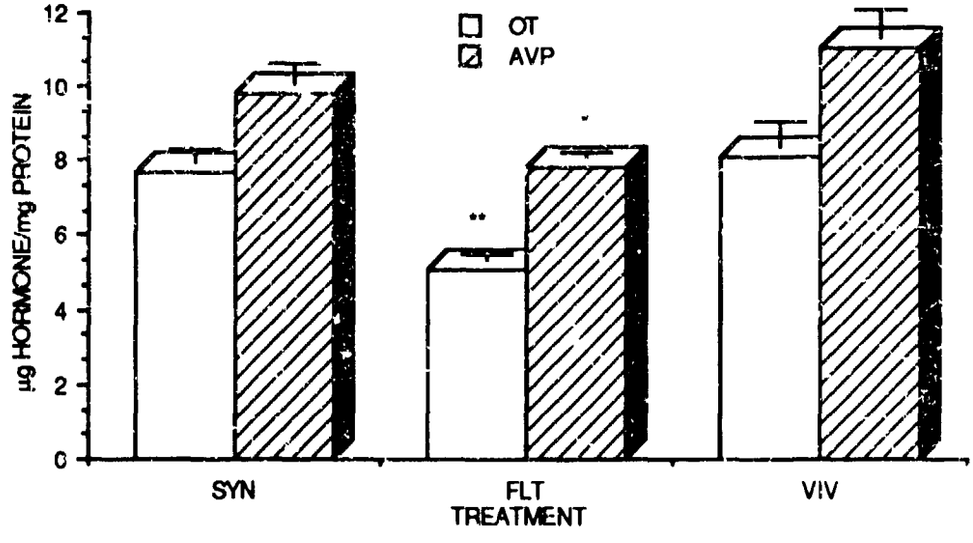

Figure 2. Oxytocin (OT) and vasopressin (AVP) content expressed as micrograms of hormone per mg of protein in the posterior pituitary extract. ** p<0.05 for comparison of Flight to either Syncronous or Vivarium control by both the ANOVA or Mann-Whitney U tests. Values are means ± SE.